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Hormonal and clinical predictors for post-egg retrieval pain in women undergoing assisted reproductive technology procedures

Authors

Pascal H. Vuilleumier [a], Emily Dinges [a], Christopher Ciliberto [a], Clemens M. Ortner [a], Paul Zarutskie [b], Ruth Landau [a]

[a] Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA

[b] Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of Washington, Seattle, WA, USA

Corresponding author

Ruth Landau, MD

Professor of Anesthesiology

Columbia University Medical Center

Columbia University College of Physicians & Surgeons

630 West 168th St PH-5

New York, NY 10032

Office: (212) 305-7412, Mobile (206) 605-4527

Email: rl262@cumc.columbia.edu

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Abstract

Objectives

The intensity of post-egg retrieval pain is underestimated, with few studies examining post-procedural pain and predictors to identify women at risk for severe pain. We evaluated the influence of pre-procedural hormonal levels, ovarian factors, as well as mechanical temporal summation (mTS) as predictors for post-egg retrieval pain in women undergoing in vitro fertilization (IVF).

Methods

Eighteen women scheduled for ultrasound-guided egg retrieval under standardized anesthesia and post-procedural analgesia were enrolled. Pre-procedural mTS, questionnaires, clinical data related to anesthesia and the procedure itself, post-procedural pain scores and pain medication for breakthrough pain were recorded. Statistical analysis included Pearson product moment correlations, Mann-Whitney U tests and multiple linear regressions.

Results

Average peak post-egg retrieval pain during the first 24 hours was 5.0 ± 1.6 on an NRS scale (0=no pain, 10=worst pain imaginable). Peak post-egg retrieval pain was correlated with basal antimullerian hormone (AMH) (r=0.549, p=0.018), pre-procedural peak estradiol (r=0.582, p=0.011), total number of follicles (r=0.517, p=0.028) and number of retrieved eggs (r=0.510, p=0.031). Ovarian hyperstimulation syndrome (OHSS) (n=4) was associated with higher basal AMH (p=0.004), higher peak pain scores (p=0.049), but not with peak estradiol (p=0.13). The mTS did not correlate with peak post-procedural pain (r=0.266, p=0.286), or peak estradiol level (r=0.090, p=0.899).

Discussion

Peak post-egg retrieval pain intensity was higher than anticipated. Our results suggest that post-egg retrieval pain can be predicted by baseline AMH, high peak estradiol, and OHSS. Further studies to evaluate intra- and post-procedural pain in this population are needed, as well as clinical trials to assess post-procedural analgesia in women presenting with high hormonal levels.

Key words:

Egg retrieval, post-procedure pain, Estrogen,

Introduction

In the Unites States, more than 150,000 assisted reproductive technology (ART) procedures were performed in 2011 [1] with numbers anticipated to further increase. Oocyte retrieval is only one of many steps and painful procedures that women will experience before becoming a parent. Women undergoing ART report escalating pelvic discomfort in the later stages of ovulation induction and most women use potent analgesics following oocyte retrieval procedures [2, 3]. Because post-procedural pain has not been extensively studied and management is challenging due to concerns that anti-inflammatory drugs may interfere with the fertility process, women are at risk for being either under or over-treated with prescribed opioids that may not necessarily be effective or even needed.

Discomfort and pain after ART has been attributed to the mechanical process of ovarian enlargement similar to midcycle pelvic pain (Mittleschmerz) [4], the operative procedure itself, and the resulting post-operative accumulation of peritoneal fluid and blood [5]. The ovulation induction protocols utilized in ART are designed to induce supraphysiologic oocyte recruitment. With continued ovulation induction, follicular recruitment results in significant bilateral ovarian enlargement from multiple follicles and supraphysiologic estradiol levels. In recent years, estradiol (E2) has been increasingly studied as a modulator of pain involved at the level of the dorsal horn and NMDA transmission [6-8]. However, the impact on pain modulation and pain perception of the supraphysiologic levels of estradiol and the overall altered endocrine state seen in ART, have not been investigated.

Quantitative sensory tests (QST) evaluate the psychophysical response to experimental noxious stimuli, allowing quantification of clinically relevant pain thresholds and tolerance [9]. Simple bedside testing of mechanical temporal summation (mTS) with a Von Frei filament has been shown to predict acute post-operative pain [12, 13] and chronic pain disorders [14-16] in clinical studies. Only a handful of studies have utilized QST to evaluate the influence of hormonal stimulation for ART on pain perception [17-19], and none has evaluated pre-procedural pain testing or hormonal assays with post-procedural pain. Increased sensitivity to thermal cutaneous pain was found in 31 women undergoing ART but only at supraphysiologic E2 levels [17]. No association between hormone levels at different time-points during hormonal stimulation and a variety of QST was found in another study in 16 women undergoing ART [18], and only reductions in cold water tolerance, but not in pressure pain were found in another study in 40 women [19].

We decided to characterize post-egg retrieval pain and analgesic consumption, as well as identify clinical predictors for severe post-procedural pain, defined as a pain score of more than 7 on a scale from 0-10. We therefore designed a prospective, observational study exploring mTS, pain scores and analgesic intake, questionnaires to assess anxiety, fear and catastrophization traits (Spielberger's State-Trait Anxiety Inventory, Pain Catastrophizing Scale, Fear of Pain, Short-form McGill Pain Questionnaire (SF-MPQ) and hormonal levels to study the association between these parameters and post-egg retrieval pain.

Materials and Methods

Ethical consent

The study was approved by the University of Washington ethics committee (No. 35811-A), registered in the Clinical Trials Protocol Registration System (NCT00867945), and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Subjects

Nulliparous women scheduled for egg retrieval after hormonal stimulation were approached the day before the egg retrieval procedure. Women were considered eligible if they were healthy, aged 18-45 years, with no prior history of anxiety, no depression or chronic pain and no chronic opioid consumption. Intake of non-steroidal anti-inflammatory drugs (NSAIDs) or acetaminophen 48 hours prior to the egg retrieval was not allowed, as per clinical protocol at the Division of ART at the University of Washington.

Hormonal assays

Prior to enrollment in the IVF cycle, antimullerian hormone (AMH), thyroid panel, prolactin, and a baseline (day 2-4 of menstrual cycle) E2 and FSH were measured in all women. E2 levels were drawn at the start of the IVF cycle, on monitored stimulation days, and on the morning after hCG administration (peak E2) (Figure 1).

Ultrasound assessments

Serial transvaginal ultrasounds were performed per clinical protocol to determine follicular growth and timing for hCG administration, as well as the total follicle number (follicles greater than 8 mm) and lead follicles (greater than 14 mm). Follicle growth was assessed by serial transvaginal ultrasounds, starting from stimulation day 5 and thereafter as often as necessary in order to ensure that the antagonist and the hCG administration met protocol. On the day of hCG administration ultrasound assessment included recording total number and mean diameters for all follicles above 8mm size. Transvaginal ultrasound oocyte retrieval was performed 36 hours after hCG administration.

Ovarian stimulation

In vitro fertilization (IVF) ovulation induction protocol (Figure 1) was initiated with oral contraceptive pretreatment. After a baseline transvaginal ultrasound, E2 and progesterone levels to confirm ovarian suppression (day 1 of a 10 day stimulation cycle), ovarian stimulation was initiated. A gonadotropin/antagonist/ovulation induction protocol was followed and employed a combination of recombinant FSH (Gonal-F™, Merck Serono) and menotropin (Menopur™, Ferring). With follicular recruitment achieving lead follicles of 12-13mm mean diameter, a GnRH antagonist (Cetrotide™, Merck Serono) was begun at a daily dose of 0.25mg. With lead follicle cohort of at least 17mm mean diameter and a total serum E2 reflective of 200-250 pg/ml per follicles, final oocyte maturation was induced by administering 10,000 IU of hCG (Noverel™, Ferring).

Egg retrieval procedure

Using a standardized technique under ultrasound guidance and with general anesthesia, the aspiration needle (OVA-Stiff, 16 GA/35 cm, Cook Medical, Brisbane, QLD, Australia) was inserted through the vaginal wall and into the ovary. In a serial fashion, follicular aspiration was performed of all follicles greater than 13mm size. To minimize the number of transvaginal/ovarian wall punctures, the needle was kept in the aspirated/collapsed follicle and advanced to the next adjacent follicle. Once all follicles in a given ovary were aspirated and oocyte identification complete, the needle was removed. The pelvis and ovary were observed with transvaginal Doppler, and if necessary hemostasis was achieved with approximated manual abdominal pressure with vaginal probe pressure. With adequate observation of hemostasis, transvaginal egg retrieval was resumed and follicular aspiration of the remaining ovary was completed. Upon completion of the transvaginal egg retrieval and a transvaginal Doppler ultrasound confirming hemostasis, anesthesia was discontinued and patients transferred to the post-anesthesia care unit (PACU).

The luteal phase was supplemented daily with vaginal progesterone (Crinone 8%, Watson) and oral E2 6mg (2mg three times a day), beginning the morning after oocyte recovery.

Anesthesia and post-egg retrieval analgesia

Anesthesia was provided by a dedicated team of obstetric anesthesiologists following a standardized protocol and in keeping with clinical standards requested by the Division of ART at the University of Washington. This protocol included intravenous (IV) midazolam 2mg, fentanyl 50-200mcg, propofol (1-2mg/kg) titrated to loss of consciousness and

maintained with a continuous infusion (50-150mcg/kg/min), ondansetron 4mg and dexamethasone 4mg. At the end of the procedure and after regaining consciousness, women were monitored in the PACU for two hours, and offered oral hydrocodone 5mg - acetaminophen 500mg. Breakthrough pain was treated with additional IV fentanyl at the discretion of the anesthesiologist. Women were instructed to manage post-egg retrieval pain with hydrocodone 5mg and acetaminophen 500mg tablets per need.

Study procedures

Written consent was obtained the morning of the procedure. Two research coordinators not involved with clinical care collected all the pre-procedural questionnaires, performed mTS and the 24 hours phone pain follow-up. The full study flow is presented in Figure 2.

- *Questionnaires:*

On the day of egg-retrieval and before any procedure-related intervention was started, women completed written questionnaires recording demographic data, general health information, as well as validated questionnaires to assess anxiety, fear and catastrophization traits (Spielberger's State-Trait Anxiety Inventory, Pain Catastrophizing Scale, Fear of Pain, Short-form McGill Pain Questionnaire (SF-MPQ) [21, 22].

- *Mechanical temporal summation (mTS):*

In sum, mTS was evoked with a 180 g (#6.45) von Frey filament (North Coast Medical, San Jose, California) applied to the volar aspect of the dominant forearm. Women were asked to report their pain intensity on a 101 - point verbal numerical response scale (NRS; 0 = no pain, 100 = worst pain imaginable) from a single stimulus pinprick. Subsequently, ten

repetitive stimuli with an inter-stimulus interval of 1 second were applied within an area of 1 cm in diameter using the same filament, and women were asked to rate the pain intensity of the last stimulus. The mTS score calculated as the difference between the last and first pain scores, with a positive score indicating increased excitatory central pain facilitation [12], as previously described [13].

- *Pain outcomes:*

  Women were contacted by phone the day after the procedure and answered questions on a scripted interview assessing their peak pain (numeral pain score 0-10; 0=no pain, 10=worst pain imaginable), analgesic consumption (tablets taken), and pain after taking pain medication (numeral pain score 0-10; 0=no pain, 10=worst pain imaginable).

Statistical Analysis

Descriptive categorical and numerical variables are presented as percentages or means ± standard deviation. P values < 0.05 were considered significant. All available variables fulfilled normal distribution criteria. Mann-Whitney *U* tests were performed, investigating the impact of ovarian hyperstimulation syndrome (OHSS) on peak E2, basal AMH levels and peak post-procedural pain. Data were analyzed using SPSS 19 (SPSS Inc; Chicago, IL).

Results

During the study period, 20 women were enrolled into the study, with 18 completing all the measures and follow-up, as described in the study flowchart (Figure 1). The overview of all demographic parameters are presented in Table 1, pain measures and the Pearson product-moment correlation coefficients are presented in Table 2.

Women received on average IV fentanyl 97.2mcg ± 29.5 during the procedure and immediately after in the PACU. For post-procedural analgesia, women took on average 16.1mg ± 13.2 oral hydrocodone and 1609mg ± 1325 oral acetaminophen in the first 24 hours.

All women experienced moderate to severe post-procedural pain (NRS peak pain score ≥ 3/10). The average peak pain score in the first 24 hours after the procedure was 5.0 ± 1.6. The average pain score after taking pain medication was 2.7 ± 1.6, which was significantly lower than the peak post-procedural pain score ($p<0.001$) (Figure 3). Pain was described as sharp (n=6), crampy (n=14) and burning (n=1). A majority of patients experienced more pain than they expected (n=11).

A Pearson product-moment correlation coefficient was computed to assess the strength of the linear association between peak post-procedural pain, demographic data, mTS, hormonal and procedural factors (number of retrieved eggs, surgical time, total follicles and lead follicles). Two separate standard multiple regressions were performed to keep a minimal number of variables in this small dataset and to avoid multicollinearity [23, 24]. A standard multiple regression analysis was conducted to assess potential correlations between age, BMI and

mTS and peak post-procedural pain. The regression equation for predicting maximal post-procedural pain was:

Peak post-procedural pain = (0.055*mTS) + (0.031 * BMI) - (0.116 * age) + 8.502.

Procedure-related factors possibly explaining the maximal pain were evaluated separately for the above stated reasons. A standard multiple regression analysis was conducted exploring the correlation of peak post-procedural pain with the number of retrieved eggs, surgical time, total follicles and lead follicles. The regression equation for predicting peak post-procedural pain was:

Peak post-procedural pain= (0.179 * retrieved eggs) + (0.086 * total follicles) – (0.270* lead follicles) + (0.002* surgical time) + 3.711

Peak post-procedural pain was significantly associated with 4 factors: basal AMH level (r=0.549, p=0.018), peak E2 level (r=0.582, p= 0.011), the total number of follicles (r=0.591, p=0.010) and the number of retrieved eggs (r=0.510, p=0.031) (Figure 4).

The linear combination of procedural factors (surgical time, number of retrieved eggs, total follicles and lead follicles) was not significantly associated with post-procedural analgesic response, defined as the difference in pain score (NRS) between the peak post-procedural pain score and average pain score (NRS) after taking pain medication (F=0.418, p=0.793, R=0.338).

Pre-procedural mTS score was 2.47 ± 4.7 (on a scale from 0-100; 0=no pain and 100= worst pain imaginable) and was neither associated with peak E2 levels (r=0.090, p=0.724), nor peak post-procedural pain (r=0.266, p=0.286). Peak E2 level was significantly associated

with basal AMH level (r=0.718, p=0.001), the total number of follicles (r=0.719, p=0.001), the number of lead follicles (r=0.638, p=0.014), and the number of retrieved eggs (r=0.831, p<0.001),

The number of retrieved eggs was correlated with basal AMH level (r=0.701, p=0.001), the total number of follicles (r=0.752, p<0.001), and lead follicles (r=0.752, p<0.001). The total number of follicles was significantly correlated with basal AMH level (r=0.591, p=0.010) and lead follicles (r=0.688, p=0.002).

The linear combination of demographic factors (age, BMI) and mTS was not significantly correlated with peak post-procedural pain, F (4,13)=1.329, R=0.471, R^2=0.222. The multiple correlation coefficient was 0.471, indicating that approximately 22% of the variance of peak post-procedural pain can be accounted for by the linear combination of the predictors.

The linear combination of procedural factors (surgical time, number of retrieved eggs, total follicles and lead follicles) was significantly correlated with peak post-procedural pain, F (4,13)=4.756, p=0.014. The multiple correlation coefficient was 0.771, indicating that approximately 60% of the variance of peak post-procedural pain can be accounted for by the linear combination of predictors, with the number of eggs (p=0.032) and the number of lead follicles (p=0.009) being statistically significant.

There were 4 cases diagnosed with OHSS, using a cut-off of AMH level above 3.5 ng/ml [25]. Mann-Whitney U tests were performed, to evaluate the impact of ovarian hyperstimulation syndrome (OHSS) on peak E2, basal AMH levels and peak post-procedural pain. Women with OHSS had significantly higher levels of AMH (6.42ng/ml ±2,3) than women without OHSS (1.72ng/ml ±1.1; z=-2.91; p=0.004; mean rank with OHSS=14.5,

without OHSS=6.5). Peak post-procedural pain scores were higher in women with OHSS (z=-1.96; p=0.049; mean rank with OHSS=12.5, without OHSS=7.17). Peak E2 did not correlate with OHSS (z=-1.57, p=0.13; mean rank with OHSS=11.75, without OHSS=7.42).

Discussion

The main findings in this study are that women undergoing ultrasound-guided egg retrievals report moderate to severe peak-procedural pain that is described by most as sharp and exceeding their expectations. In addition, basal AMH levels and peak E2 levels were found to correlate with peak post-procedural pain, and may be used as predictors for severe pain in women with high pre-procedural hormonal levels. Basal AMH levels have been associated with the total number of follicles on the day of procedure, peak E2 levels and number of retrieved eggs [26], and are used as a predictor for follicular recruitment and influence medication protocols for adjusting gonadotropin stimulation [27, 28], but we provide here novel information suggesting it is also associated with the intensity of post-procedural pain. This association of basal AMH level and peak post-egg retrieval pain was not expected. Basal AMH levels AMH levels above 3.5 ng/ml have been associated with ovarian hyperstimulation [25]; and in our cohort, 4 women met criteria for OHSS.

If ovarian distention is the main contributor to post-procedural pain, it would be expected that both the total number of recruited follicles as well as the number of lead follicles are associated with post-procedural pain. In our study, only the total number of recruited follicles and the number of retrieved eggs, but not the number of lead follicles, correlated in a multiple regression analysis predicting peak post-procedural pain. Our data suggests that the altered endocrine state seen in women undergoing fertility treatment contributes to post

sonographic egg recovery pain modulation: in particular the hormonal contribution related to the supraphysiologic levels of estradiol seen in ART in contrast to mechanical, anatomical or procedure-related factors, are causation for the post procedural pain. The exact implications of this finding remains to be established, and may be limited to our small sample.

The intensity of pain reported by our cohort of women is substantially higher than that reported in a recent systematic review [5]. Explanations for this finding may be related to several factors, including the fact that women in our study did not receive regional anesthesia or local anesthetics, were not allowed to take NSAIDs before or after the procedure, and were given acetaminophen and hydrocodone-based tablets, which may not be as effective for crampy pain as other analgesic/anesthetic modalities. In addition, with 4 cases of OHSS (22% of our cohort), higher pain scores can be anticipated as we and others [19] found that supraphysiological levels of E2 are associated with higher sensitivity to pain.

In a recent study evaluating symptoms such as abdominal pain and cramping, bloating, headaches, nausea, increased thirst, sweating, hunger, anxiety, irritability, vaginal discomfort and fatigue in 51 women undergoing IVF and 12 healthy controls, peak E2 levels were temporally associated with peak physical symptoms in the IVF group, supporting the hypothesis that increasing E2 levels influence symptom severity [3].

We evaluated pre-operatively mTS, as this simple test has been previously shown to be associated with acute post-operative pain [12], and we hypothesized that in the context of high estrogen impregnation, increased ascending noxious input may result in abnormal wind-up of pain. We did not find any correlation between mTS values and any of the outcomes. The lack of association with any of the measures outcomes and specifically with peak-

procedural pain is most likely due to the fact that women in our cohort were all healthy, with no history of chronic pain, and were found to all have extremely low scores of mTS, therefore this test could not serve as a predictor in this setting. Alternatively, mTS may not be the most specific QST to predict pain that is essentially crampy by nature. Based on our findings, we can only conclude that mTS scores in otherwise healthy women do not seem to increase with the endocrine state induced by IVF.

We are aware of several limitations in our study design, which require cautious interpretation of our findings. First, we acknowledge our small sample size, which was in part due to our desire to ensure the most standardized hormonal stimulation protocol and procedure technique, so we only enrolled cases performed by one specific ART provider. Second, women did not receive NSAIDs (as per protocol established by the ART provider), which resulted in relatively high pain scores in all women. Last, we did not examine the effect of elevated AMH levels and peak E2 on pain perception after different procedures or in a context that is not related with egg retrievals, therefore more studies will be needed to establish the correlation and potential mechanisms by which these two hormones may be negatively influencing pain perception in women.

In sum, to the best of our knowledge, this is the first study evaluating pre-procedural hormonal levels, mechanical temporal summation, and procedural factors in the context of post egg-retrieval pain and analgesic consumption. Although the sample size is small and results have to be interpreted with caution, our findings are consistent and provide robust evidence for a correlation between basal AMH levels, peak E2 levels and the intensity of post

egg-retrieval pain. These findings linking hormonal levels to pain merit further investigation to improve post-procedural management.

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References

1. CDC. 2011 ART Report [Web Page]. 2011. Available at http://www.cdc.gov/art/ART2011.

2. Gejervall AL, Stener-Victorin E, Cerne A, Borg K and Bergh C. Pain aspects in oocyte aspiration for IVF. *Reproductive Biomedicine online* 2007;14:184-90.

3. Suthersan D, Kennedy S and Chapman M. Physical symptoms throughout IVF cycles. *Human Fertility* 2011;14:122-8.

4. Won HR and Abbott J. Optimal management of chronic cyclical pelvic pain: an evidence-based and pragmatic approach. *International Journal of Women's Health* 2010;2:263-77.

5. Kwan I, Bhattacharya S, Knox F and McNeil A. Pain relief for women undergoing oocyte retrieval for assisted reproduction. *The Cochrane Database of Systematic Reviews* 2013;1:CD004829.

6. Tang B, Ji Y and Traub RJ. Estrogen alters spinal NMDA receptor activity via a PKA signaling pathway in a visceral pain model in the rat. *Pain* 2008;137:540-9.

7. Zhang Y, Lu N, Zhao ZQ and Zhang YQ. Involvement of estrogen in rapid pain modulation in the rat spinal cord. *Neurochemical Research* 2012;37:2697-705.

8. Zhang Y, Xiao X, Zhang XM, Zhao ZQ and Zhang YQ. Estrogen facilitates spinal cord synaptic transmission via membrane-bound estrogen receptors: implications for pain hypersensitivity. *The Journal of Biological Chemistry* 2012;287:33268-81.

9. Werner MU, Petersen MA and Bischoff JM. Test-retest studies in quantitative sensory testing: a critical review. *Acta Anaesthesiologica Scandinavica* 2013;57:957-63.

10. Nielsen J and Arendt-Nielsen L. Spatial summation of heat induced pain within and between dermatomes. *Somatosensory & Motor Research* 1997;14:119-25.

11. Baranauskas G and Nistri A. Sensitization of pain pathways in the spinal cord: cellular mechanisms. *Progress in Neurobiology* 1998;54:349-65.

12. Weissman-Fogel I, Granovsky Y, Crispel Y, Ben-Nun A, Best LA, Yarnitsky D and Granot M. Enhanced presurgical pain temporal summation response predicts post-thoracotomy pain intensity during the acute postoperative phase. *The Journal of Pain* 2009;10:628-36.

13. Ortner CM, Granot M, Richebe P, Cardoso M, Bollag L and Landau R. Preoperative scar hyperalgesia is associated with post-operative pain in women undergoing a repeat Caesarean delivery. *Eur J Pain* 2013;17:111-23.

14. Fillingim RB, Fillingim LA, Hollins M, Sigurdsson A and Maixner W. Generalized vibrotactile allodynia in a patient with temporomandibular disorder. *Pain* 1998;78:75-8.

15. George SZ, Wittmer VT, Fillingim RB and Robinson ME. Fear-avoidance beliefs and temporal summation of evoked thermal pain influence self-report of disability in patients with chronic low back pain. *Journal of Occupational Rehabilitation* 2006;16:95-108.

16. George SZ, Wittmer VT, Fillingim RB and Robinson ME. Sex and pain-related psychological variables are associated with thermal pain sensitivity for patients with chronic low back pain. *The Journal of Pain :* 2007;8:2-10.

17. Nisenblat V, Engel-Yeger B, Ohel G, Aronson D and Granot M. The association between supra-physiological levels of estradiol and response patterns to experimental pain. *Eur J Pain* 2010;14:840-6.

18. Stening KD, Berg G, Hammar M, Voster H, Eriksson O, Amandusson A and Blomqvist A. Influence of estrogen levels on thermal perception, pain thresholds, and pain tolerance: studies on women undergoing in vitro fertilization. *The Journal of Pain* 2012;13:459-66.

19. Tsen LC, Natale M, Datta S and Eappen S. Can estrogen influence the response to noxious stimuli? *Journal of Clinical Anesthesia* 2001;13:118-21.

20. Wolters U, Wolf T, Stutzer H and Schroder T. ASA classification and perioperative variables as predictors of postoperative outcome. *British Journal of Anaesthesia* 1996;77:217-22.

21. Kalkman CJ, Visser K, Moen J, Bonsel GJ, Grobbee DE and Moons KG. Preoperative prediction of severe postoperative pain. *Pain* 2003;105:415-23.

22. Pavlin DJ, Sullivan MJ, Freund PR and Roesen K. Catastrophizing: a risk factor for postsurgical pain. *The Clinical Journal of Pain* 2005;21:83-90.

23. Stevens JP. *Applied Multivariate Statistics for the Social Sciences*. Psychology Press, 2002, 2002.

24. Tabachnick BG, Fidell, L.S. *Using Multivariate Statistics*. Pearson; 6 edition (June 25, 2012), 2012.

25. Toner JP and Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimullerian hormone. *Fertility and Sterility* 2013;99:1825-30.

26. Patrelli TS, Gizzo S, Sianesi N, Levati L, Pezzuto A, Ferrari B and Bacchi Modena A. Anti-Mullerian hormone serum values and ovarian reserve: can it predict a decrease in fertility after ovarian stimulation by ART cycles? *PloS one* 2012;7:e44571.

27. Li HW, Lee VC, Ho PC and Ng EH. Ovarian sensitivity index is a better measure of ovarian responsiveness to gonadotrophin stimulation than the number of oocytes during in-vitro fertilization treatment. *Journal of Assisted Reproduction and Genetics* 2013.

28. La Marca A and Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. *Human Reproduction Update* 2014;20:124-140.

Figure legends

Figure 1. Study Flowchart

Figure 2. Study protocol

The entire IVF ovulation induction protocol is represented, with 10-21 days of pretreatment cycle, 10 days of stimulation cycle, 2 days of maturation cycle, the procedure and following 24 hours.

The study protocol included analysis of baseline E2, FSH, AMH levels, peak E2 levels, questionnaires, mTS, procedure related parameters, as well as by pain scores during the first 24 hours (all study measures are represented in bold red).

E2 = estradiol

FSH = Follicle Stimulating hormone

AMH = Antimullerian hormone

IVF = In Vitro Fertilization

GnRH = gonadotropin-releasing hormone

hCG = human chorionic gonadotropin

mTS = mechanical temporal summation

Figure 3. Pain outcomes

Peak pain post-egg retrieval was the worst pain during the first 24 hour after the procedure (presented as 18 individual scores, with a numerical rating scale from 0-10; 0=no pain, 10=worst pain imaginable).

Pain after taking pain medication was the pain intensity women reported after taking pain medication (presented as 18 individual scores, with a numerical rating scale from 0-10; 0=no pain, 10=worst pain imaginable).

Figure 4. Correlations between peak E2, baseline AMH, total number of follicles and number of retrieved eggs with peak post-procedural pain

Correlations performed using Pearson correlation coefficient

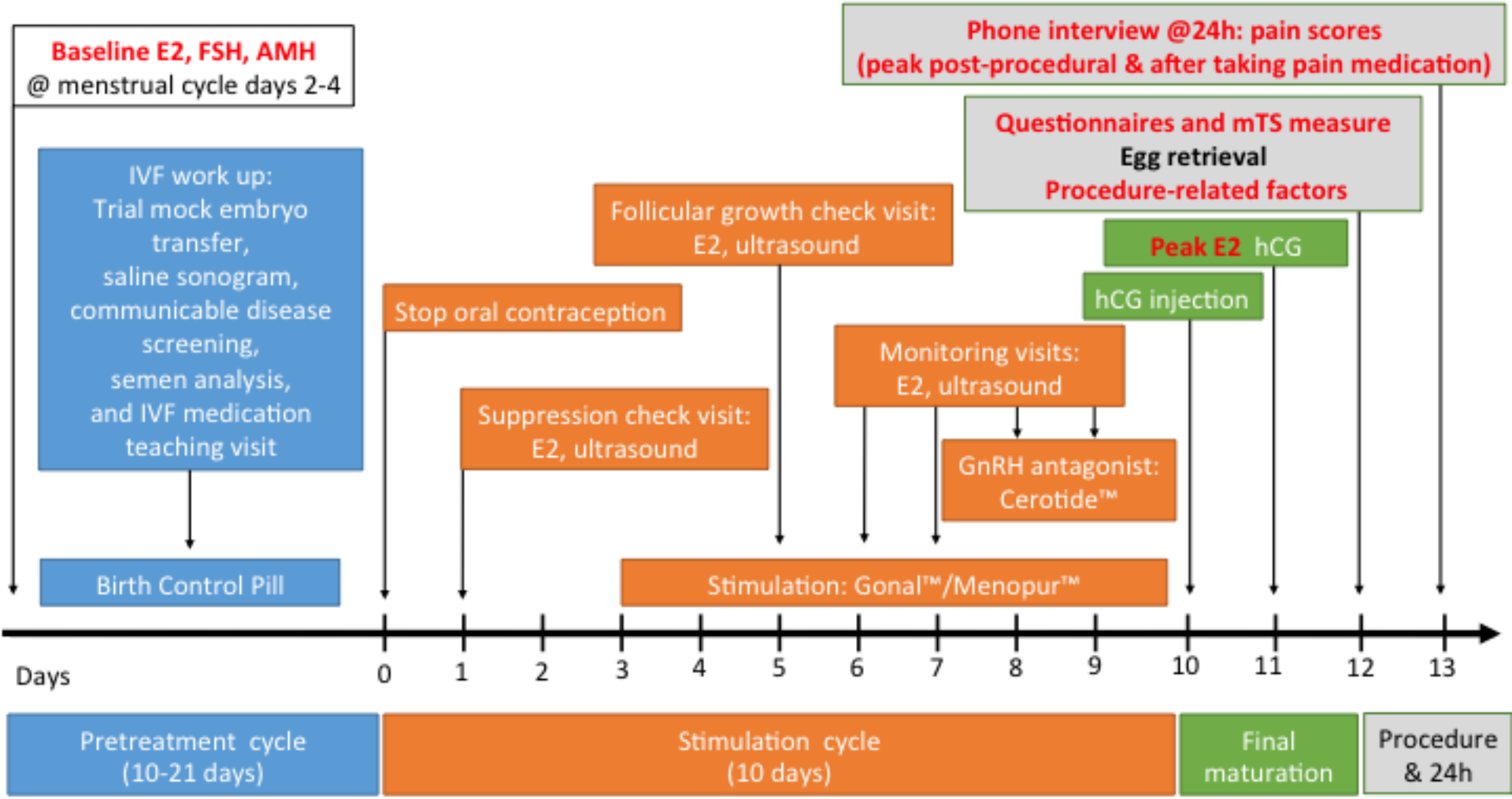
Figure 4A. Peak estradiol level correlated with peak pain post-egg retrieval (representing the worst pain during the first 24 hour after the procedure)

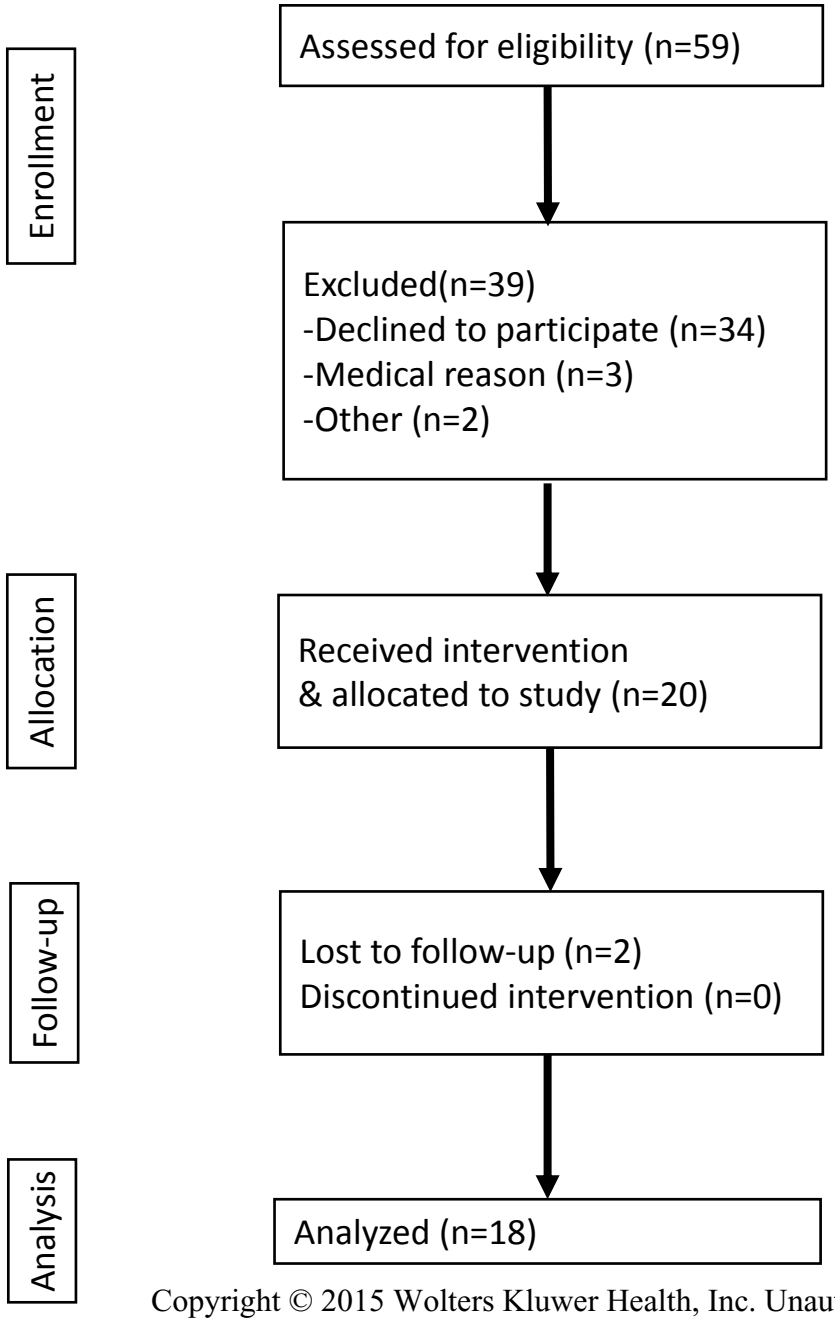
Figure 4B. Baseline AMH level correlated with peak pain post-egg retrieval (representing the worst pain during the first 24 hour after the procedure)

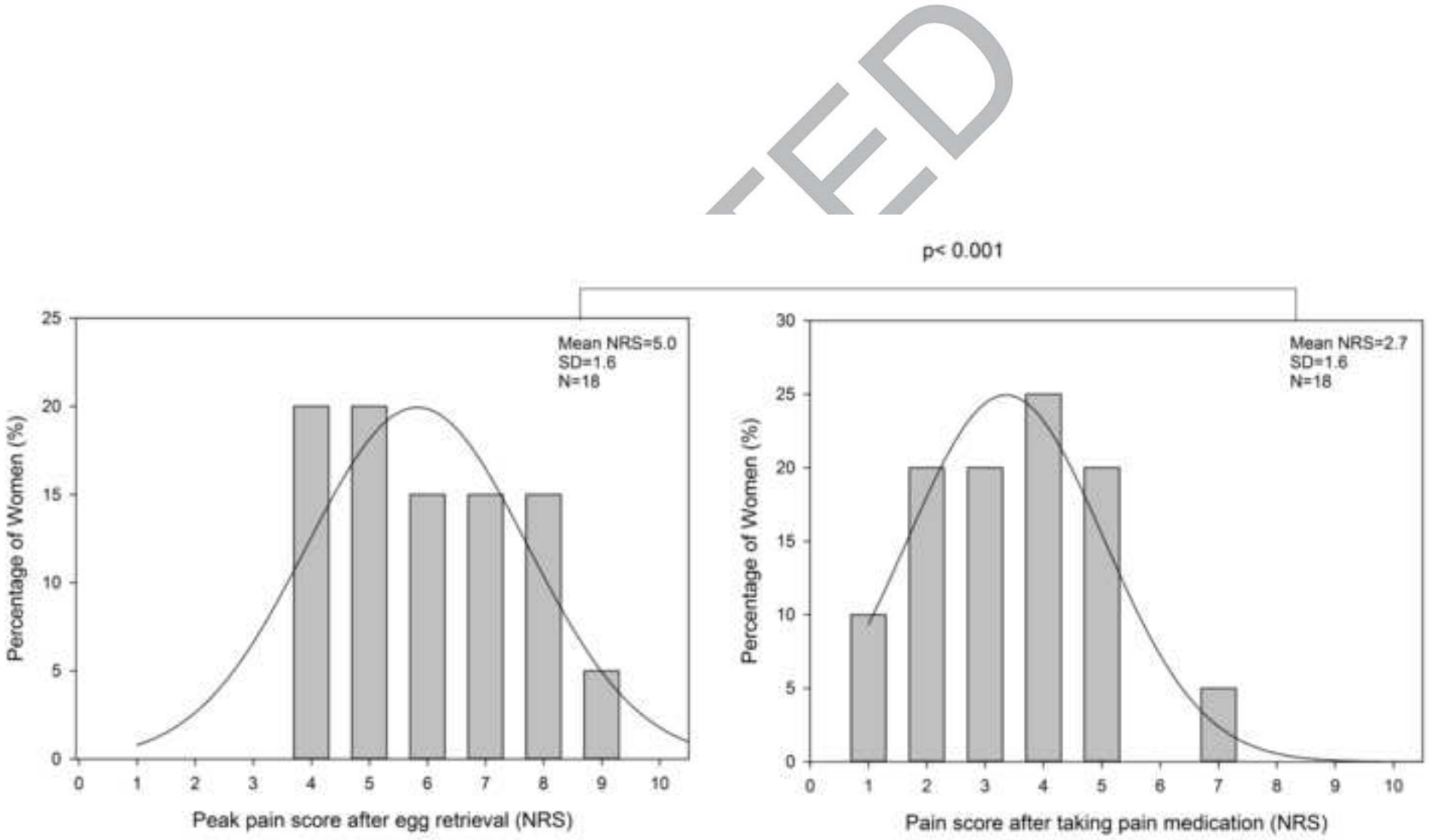
Figure 4C. Number of total follicles correlated with peak pain post-egg retrieval (representing the worst pain during the first 24 hour after the procedure)

Figure 4D. Number of retrieved eggs correlated with peak pain post-egg retrieval (representing the worst pain during the first 24 hour after the procedure)

AMH = Antimullerian hormone







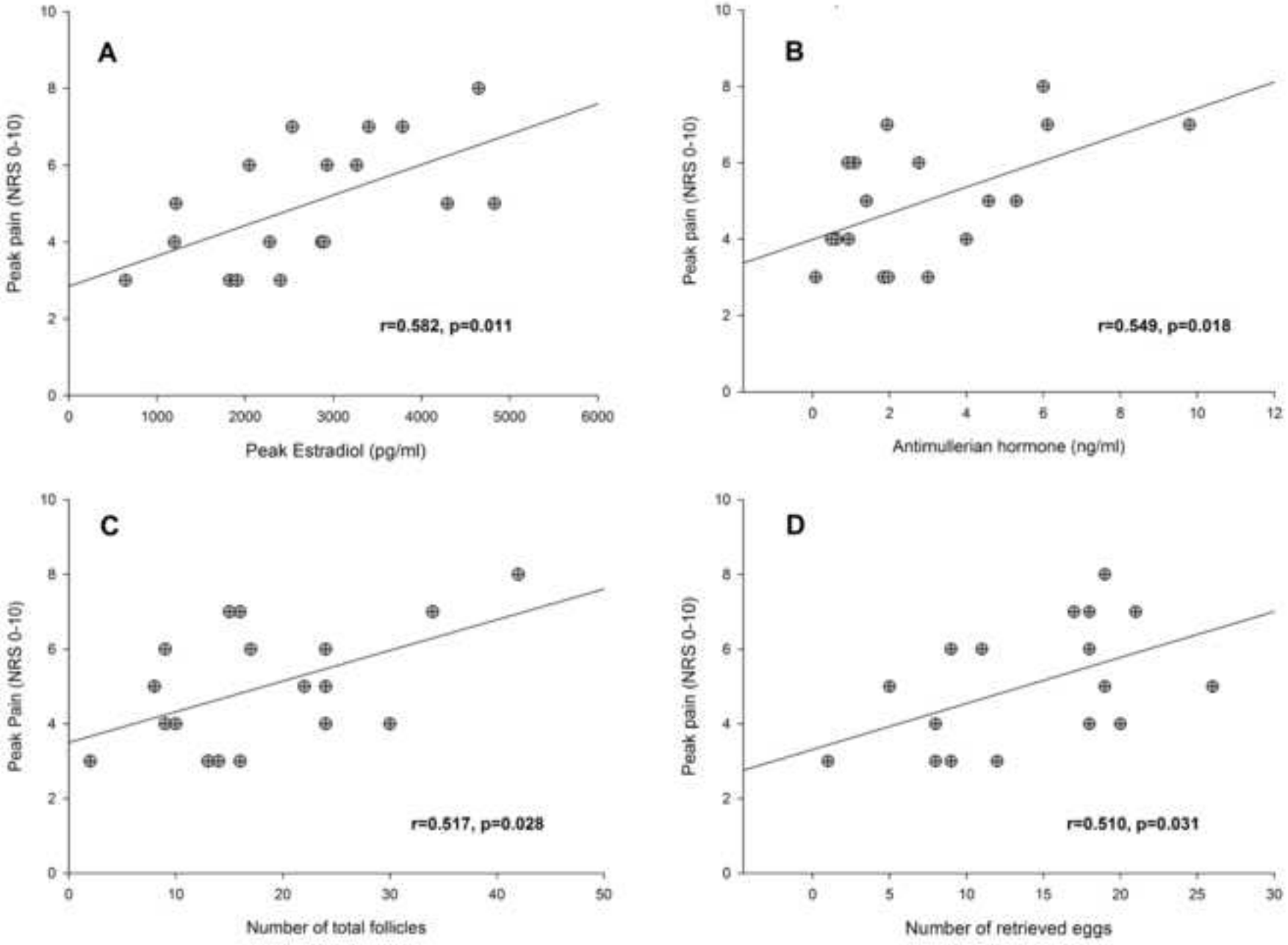


Table 1. Demographic variables

Age (years)	36.1 (±5.6)
Weight (kgs)	68.1 (±18.8)
BMI (kgs/m^2)	24.8 (±5.9)
Peak E2 (pg/ml)	2720 (±1183)
mTS score (0-10)	2.47 (±4.1)
AMH (ng/ml)	2.9 (±2.6)
E2 @ day 3 (pg/ml)	49.6 (±27.4)
FSH @ day 3 (mIU/ml)	7.6 (±4.2)
Procedure duration (min)	22.78 (±9.9)
Fentanyl dose (mcg)	97.2 (±29.57)
Retrieved eggs (N)	13.7 (±6.6)
Total follicles (N)	18.3 (±10.1)
Lead follicles (N)	10.3 (±5.1)
Peak pain (NRS 0-10)	5.0 (±1.6)
Hydrocodone (mg)	16.1 (±13.2)
Paracetamol (mg)	1609 (±1325)

Data presented as mean ± standard deviation (SD).
Peak pain = peak post-procedural pain (NRS scale from 0-10)
Peak E2 = maximal plasmatic level measured after hormonal stimulation
AMH = Antimullerian hormone,
E2 = plasmatic estradiol between days 2 -5
FSH = Follicle-stimulating hormone between days 2-5
mTS = mechanical temporal summation
Hydrocodone = analgesic intake of oral hydrocodone in the first 24h
Paracetamol = analgesic intake of oral paracetamol in the first 24h

Table 2. Pearson correlation matrix

Variable	Age	Weight	BMI	Procedure duration	Fentanyl	Peak pain	Peak E2	Retrieved eggs	Total follicles	Lead follicles	AMH	E2 @day 3	FSH @day 3	mTS	Hydrocodone
Weight	r=0.241, p=0.335	x													
BMI	r=0.310, p=0.210	r=0.957, p<0.001*	x												
Procedure duration	r=-0.425, p=0.079	R=-0.398, p=0.101	r=-0.452, p=0.059	x											
Fentanyl	r=-0.002, p=0.994	R=0.257, p=0.303	r=0.295, p=0.235	r=-0.380, p=0.120	x										
Peak pain	r=-0.427, p=0.077	r=0.072, p=0.775	r=0.006, p=0.982	r=0.285, p=0.251	r=0.247, p=0.322	x									
Peak E2	r=-0.359, p=0.144	r=-0.106, p=0.667	r=-0.134, p=0.059	r=0.274, p=0.272	r=0.171, p=0.498	r=0.582, p=0.011*	x								
Retrieved eggs	r=-0.465, p=0.052	r=-0.098, p=0.699	r=0.152, p=0.548	r=0.417, p=0.085	r=-0.138, p=0.584	r=0.510, p=0.031*	r=0.831, p=<0.001*	x							
Total follicles	r=-0.523, p=0.026*	r=-0.291, p=0.241	r=-0.443, p=0.065	r=0.591, p=0.010*	r=-0.263, p=0.292	r=0.517, p=0.028*	r=0.719, p=0.001*	r=0.752, p=<0.001*	x						
Lead follicles	r=-0.478, p=0.045*	r=-0.279, p=0.262	r=-0.338, p=0.170	r=0.415, p=0.087	r=-0.198, p=0.431	r=0.079, p=0.756	r=0.638, p=0.014*	r=0.752, p=<0.001*	r=0.688, p=0.002*	x					
AMH	r=-0.421, p=0.082	r=-0.166, p=0.511	r=-0.162, p=0.520	r=0.396, p=0.104	r=-0.168, p=0.506	r=0.549, p=0.018*	r=0.718, p=0.001*	r=0.701, p=0.001*	r=0.591, p=0.010*	r=0.323, p=0.191	x				
E2 @day 3	r=0.150, p=0.553	r=-0.131, p=0.604	r=-0.195, p=0.437	r=0.073, p=0.775	r=0.057, p=0.823	r=0.165, p=0.512	r=0.442, p=0.066	r=0.194, p=0.441	r=0.371, p=0.129	r=0.332, p=0.178	r=0.256, p=0.306	x			
FSH @day 3	r=0.450, p=0.061	r=-0.354, p=0.150	r=-0.234, p=0.350	r=-0.252, p=0.313	r=0.050, p=0.844	r=-0.315, p=0.203	r=-0.152, p=0.546	r=-0.161, p=0.524	r=-0.355, p=0.149	r=-0.110, p=0.663	r=-0.199, p=0.428	r=0.139, p=0.581	x		
mTS	r=-0.244, p=0.330	r=0.220, p=0.381	r=0.171, p=0.498	r=0.048, p=0.849	r=0.078, p=0.759	r=0.266, p=0.286	r=0.090, p=0.724	r=0.032, p=0.899	r=-0.060, p=0.812	r=0.016, p=0.951	r=-272, p=0.275	r=-0.233, p=0.352	r=-0.069, p=0.786	x	
Hydrocodone	r=-0.333, p=0.227	r=-0.162, p=0.520	r=-0.202, p=0.421	r=0.291, p=0.242	r=-0.079, p=0.755	r=0.251, p=0.315	r=0.084, p=0.738	r=0.288, p=0.246	r=0.131, p=0.605	r=-0.039, p=0.876	r=0.548, p=0.018*	r=-0.045, p=0.859	r=-0.186, p=0.474	r=-0.288, p=0.246	x
Paracetamol	r=-0.287, p=0.249	r=-0.191, p=0.448	r=-0.229, p=0.360	r=0.307, p=0.216	r=-0.077, p=0.760	r=0.202, p=0.421	r=0.075, p=0.767	r=0.266, p=0.287	r=0.116, p=0.647	r=-0.044, p=0.862	r=0.547, p=0.018*	r=-0.042, p=0.866	r=-0.207, p=0.425	r=-0.326, p=0.187	r=0.984, p<0.001*

R = Pearson correlation coefficient
* = p<0.05 (2-tailed)
Peak pain = peak post-procedural pain on a NRS scale (0-10)
Peak E2 = maximal plasmatic level measured after hormonal stimulation (pg/ml)
AMH = Antimullerian hormone (ng/ml)
E2 = peak plasmatic estradiol between days 2 -5 (pg/ml)
FSH = peak follicle-stimulating hormone between days 2-5 (mlU/ml)
mTS = mechanical temporal summation score (0-100)
Hydrocodone = analgesic intake of hydrocodone in the first 24h (mg)
Paracetamol = analgesic intake of paracetamol in the first 24h (mg)